

CLAIMS:

1. A method of providing a patient having impaired cartilage in an organ at a target site, with corresponding viable cartilage, the method comprising:
 - 5 (a) providing a receptacle containing viable cartilage having shape and size compatible with the target site in the organ in a cryopreservation solution at a temperature above the freezing temperature of the cryopreservation solution;
 - (b) cooling said viable cartilage to a temperature below said freezing temperature at a cooling rate of 0.01°C/min to 3°C/min, thereby
10 generating frozen corresponding viable cartilage.
2. The method of Claim 1, wherein the cooling in step (b) is performed by moving the receptacle along one or more consecutive temperature gradients ranging from a temperature above said freezing temperature to a temperature below said freezing temperature.
- 15 3. The method of any one of Claims 1 to 2 wherein step (b) comprises controlled initiation of seeding of freezing.
4. The method of anyone of Claims 1 to 3, comprising after step (b):
 - (c) transferring the receptacle to storage at a temperature below the freezing point of said cryopreservation solution.
- 20 5. The method of Claim 4, wherein said storage is in liquid nitrogen (LN).
6. The method of any one of Claims 1 to 5, wherein the viable cartilage comprises osteochondral tissue.
7. Frozen viable cartilage obtainable by performing the method of anyone of Claims 1 to 6.

- 28 -

8. The method of any one of Claims 1-7, comprising thawing said frozen viable cartilage, wherein said frozen viable cartilage is at an initial temperature below the glass transition temperature the method comprising:

5 (d) warming said viable cartilage from said initial temperature to an intermediate temperature being at least about said glass transition temperature or above said glass transition temperature but no more than the temperature wherein recrystallization would begin to occur at any point in the cartilage;

10 (e) warming said viable cartilage from said intermediate temperature to a temperature that is at least substantially equal to the melting temperature of the solution, said warming being at a rate sufficiently high to minimize recrystallization; thereby obtaining thawed viable cartilage.

9. The method of Claim 8, wherein said warming in step (d) is at a rate sufficiently slow to minimize fracture of said viable cartilage.

15 10. The method of anyone of Claims 8 to 9, wherein said warming in step (e) is at a rate of between 50°C/min and 1000°C/min.

11. The method of any one of Claims 8 to 10, wherein said intermediate temperature is less than -10°C, or -20 to -80°C, or -40 to -80°C or -50 to -70°C.

20 12. The method of any one of Claims 8-11, wherein said warming in step (e) comprises:

(i) removing said viable cartilage from said receptacle;

(ii) contacting the said viable cartilage with an environment having a temperature of 0°C or more.

25 13. The method of Claim 12, wherein the temperature of said environment is at least 22°C, 37°C, 50°C or at least 70°C.

- 29 -

14. The method of any one of Claims 12 to 13, wherein said viable cartilage is connected to a pulling member and the removing of step (i) is executed by pulling on said pulling member.

5 15. The method of Claim 14, wherein said pulling member is attached to the bone portion of said viable cartilage via a screw.

16. Thawed viable cartilage obtainable by performing the method of anyone of Claims 8 to 15.

10 17. Thawed viable cartilage of Claim 16, wherein the frozen viable cartilage being used in the method is obtainable by performing the steps of anyone of Claims 1 to 6.

18. The method of any one of 8-15 comprising:

(f) grafting said thawed viable cartilage in said target site.

19. The method of Claim 18, wherein said organ is a joint.

20. The method of Claim 19, wherein said joint is a knee.

15 21. The method of anyone of Claims 18 to 20, wherein the thawed viable cartilage comprises osteochondral tissue.

22. The method of Claim 21, wherein the osteochondral tissue is an osteochondral cylinder.

20 23. The method of anyone of Claims 18-22, wherein said target site is a cavity produced by drilling in the organ.

24. A method for the generation of frozen viable cartilage, said method comprising:

- 30 -

(a) providing a receptacle containing viable cartilage in a cryopreservation solution at a temperature above the freezing temperature of the cryopreservation solution;

5 (b) cooling said viable cartilage to a temperature below said freezing temperature at a cooling rate of $0.01^{\circ}\text{C}/\text{min}$ to $3^{\circ}\text{C}/\text{min}$, thereby generating frozen viable cartilage.

25. The method of Claim 24, wherein the cooling in step (b) is performed by moving the receptacle along one or more consecutive temperature gradients ranging from a temperature above said freezing temperature to a temperature below said
10 freezing temperature.

26. The method of Claim 25, wherein the velocity of movement along the at least one temperature gradient in step (b) is between $0.002\text{ mm}/\text{sec}$ and $5\text{ mm}/\text{sec}$.

27. The method of any one of Claims 24 to 25, wherein at least one of the one or more consecutive temperature gradients in step (b) is between $0.1^{\circ}\text{C}/\text{mm}$ to
15 $50^{\circ}\text{C}/\text{mm}$.

28. The method of any one of Claims 24 to 27 wherein step (b) comprises controlled initiation of seeding of freezing.

29. The method of anyone of Claims 24 to 28, comprising after step (b):

20 (c) transferring the receptacle to storage at a temperature below the freezing point of said cryopreservation solution.

30. The method of Claim 29, wherein said storage is in liquid nitrogen (LN).

31. The method of any one of Claims 24 to 30, wherein the viable cartilage comprises osteochondral tissue.

- 31 -

32. Frozen Cartilage obtainable by performing the method of anyone of Claims 24 to 31.

33. A method for thawing frozen viable cartilage that was frozen in a solution, the method comprising:

- 5 **(a)** providing a receptacle containing frozen viable cartilage at an initial temperature below the glass transition temperature of the solution;
- (b)** warming said viable cartilage from said initial temperature to an intermediate temperature being at least about said glass transition temperature or above said glass transition temperature but no more than
10 the temperature wherein recrystallization would begin to occur at any point in the cartilage;
- (c)** warming said viable cartilage from said intermediate temperature to a temperature that is at least substantially equal to the melting temperature of the solution, said warming being at a rate sufficiently high to minimize
15 recrystallization; thereby obtaining thawed viable cartilage.

34. The method of Claim 33, wherein said warming in step (b) is at a rate sufficiently slow to minimize fracture of said viable cartilage.

35. The method of Claim 34, wherein said warming in step (b) is at a rate of between 0.1°C/min and 200°C/min.

20 **36.** The method of Claim 35, wherein said warming in step (b) is at a rate of 90°C/min.

37. The method of anyone of Claims 33 to 36, wherein said warming in step (c) is at a rate of between 50°C/min and 1000°C/min.

25 **38.** The method according to Claim 37, wherein said warming in step (c) is at a rate of 200°C/min.

- 32 -

39. The method of any one of Claims 33 to 38, wherein said intermediate temperature is less than -10°C, or -20 to -80°C, or -40 to -80°C or -50 to -70°C.

40. The method of any one of Claims 33-39, wherein said warming in step (c) comprises:

- 5 (i) removing said viable cartilage from said receptacle;
- (ii) contacting the said viable cartilage with an environment having a temperature of 0°C or more.

41. The method of Claim 40, wherein the temperature of said environment is at least 22°C, 37°C, 50°C or at least 70°C.

- 10 **42.** The method of any one of Claims 40 to 41, wherein said viable cartilage is connected to a pulling member and the removing of step (i) is executed by pulling on said pulling member.

43. The method of Claim 42, wherein said pulling member is attached to the bone portion of said viable cartilage via a screw.

- 15 **44.** Thawed viable cartilage obtainable by performing the method of anyone of Claims 33 to 43.

45. Thawed viable cartilage of Claim 44, wherein the frozen viable cartilage being used in the method is obtainable by performing the steps of anyone of Claims 24 to 31.

- 20 **46.** A method of providing a patient having impaired cartilage in an organ, with corresponding thawed viable cartilage at a target site, the method comprising:

- (a) providing thawed viable cartilage of any one of Claims 44 and 45 having shape and size compatible with the target site in the organ;
- (b) grafting said thawed viable cartilage in said target site.

- 33 -

- 47.** The method of Claim 46, wherein said organ is a joint.
- 48.** The method of Claim 47, wherein said joint is a knee.
- 49.** The method of anyone of Claims 46 to 48, wherein the thawed viable cartilage comprises osteochondral tissue.
- 5 **50.** The method of Claim 49, wherein the osteochondral tissue is an osteochondral cylinder.
- 51.** The method of anyone of Claims 46-50, wherein said target site is a cavity produced by drilling in the organ.